

## STANDARD OPERATING PROCEDURE (SOP)

### METHANE BUBBLING THROUGH METHANOTROPHIC BACTERIA

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SOP #	Methane bubbling through methanotrophic bacteria
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#### 1. INTRODUCTION/PURPOSE

This is a standard procedure for methane bubbling through methanotrophic bacteria. Methanotrophic bacteria derive their carbon from methane, CH<sub>4</sub>. In order to measure the activity of these bacteria, methane must be bubbled through the culture and measured. This is done by releasing a fixed amount of methane gas from a tank into a closed system, after which the methane is pumped from a canister through tubes into the bacterial culture and back. By measuring methane vol/conc at t<sub>0</sub> and measuring after the gas has gone several rounds through the system, we can assess the methane metabolizing potential of the bacteria.

#### 2. SAFETY

General laboratory safety applies. For more information see IBV's HSE webpages: <http://www.mn.uio.no/ibv/om/hms/>. Key procedures for working in the laboratories are listed in HSE in the laboratories: <http://www.mn.uio.no/ibv/english/about/hss/laboratorier/>

**Overall risk for this SOP is**

 <b>Risk assessment of this procedure</b>	There is a <b>medium</b> overall risk associated with the use of this procedure, provided that this procedure is followed.
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#### 3. NECESSARY SAFETY EQUIPMENT

The user of the SOP should wear lab coat and gloves. This procedure should be carried out in a fume hood.

#### 4. RESPONSIBILITIES

The head of the department has overall responsibility for systematic HSE management. He/She must ensure that the section leaders who report to them, and their staff, have adequate training in HSE.

Employees shall participate in the implementation of HSE, and in accordance with the orders and instructions of IBV. Employees shall be informed of their duty to actively contribute to systematic HSE management. They shall help prevent and limit injuries and ensure work is stopped when it cannot continue without risk to life and health. Injury or illness which they believe may be due to the working environment shall be reported to the employer/manager.

#### 5. EQUIPMENT, MATERIALS AND SOLUTIONS

- Methane tank w/ valve
- Canister
- Tubing
- 250/500 mL Erlenmeyer flasks
- Pipette and pipette tips
- Methylococcus capsulatus bacterial culture (or other methanotroph)
- Methylococcus capsulatus growth medium (or for other methanotroph)
- Incubator
- Fume hood
- Peristaltic pump
- Filter for bubbling through medium
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## 6. PROCEDURES:

We always wear lab coat when working in the lab.

**Specific for this procedure use fumehood and gloves.**

*This is a general procedure for methane bubbling through methanotrophic bacteria. The risk associated with growth and expression of cells are described in separate SOPs.*

*The waste disposal is described under 7. WASTE DISPOSAL*

### **Before starting:**

*Culture M. capsulatus (or other methanotroph) in appropriate medium w/ methane, keep at 4 C.*

- Inoculate medium with bacterium*
- Fill filter with medium*
- Draw methane from tank*
- Inject methane gas into canister.*  
*Less than 500 mL; methane is flammable at 5-15 %, volume of fume hood far exceeds 10 L, therefore methane concentration is less than 5 %.*
- Depressurization step.*
- Attach peristaltic pump and activate. Incubation takes place in a small incubator in a fume hood.*
- After x minutes, measure methane left in headspace/tubing.*

## 7. RISK ASSESSMENT

The general risk factor of a SOP can be calculated using the part of the procedure with the assumed highest risk factor. The risk assessment associated with this SOP is based on the user following the precautions stated in the step by step risk assessment below.

### **List of chemicals and their H and P phrases**

Chemicals	Hazard symbol	H phrases	P phrases Precautions
Methane gas		H220 - Extremely flammable gas. H280 - Contains gas under pressure; may explode if heated. Asphyxiant in high concentrations.	P210 - Keep away from heat, sparks, open flames or hot surfaces. – No smoking. P377 - Leaking gas fire: Do not extinguish, unless leak can be stopped safely. P381 - Eliminate all ignition sources if safe to do so. P403 - Store in a well-ventilated place.

### Risk assessment; step by step

Part of procedure		Unwanted scenarios	Precautions	Emergency planning	S*K
1	Depressurization	Release of methane gas; fire/explosion hazard	Fume hood; valve; no open flames	See P-statements above	1*1(green)
2	Injecting methane into canister	Release of methane gas; fire/explosion hazard	Fume hood; valve; no open flames	See P-statements above	1*1(green)
4	Attaching and activating peristaltic pump	Break in tubing/tubing coming off attachments – Release of methane gas; fire/explosion hazard	Fume hood; valve; no open flames	-	1*1(green)

### Overall risk assessment for this SOP

If procedure specified above is followed there is MINIMAL RISK associated with the use of this SOP. If you get a yellow part of the procedure, new precautions must be assessed. If you get a red part of the procedure it will have to be changed.

### 8. WASTE DISPOSAL

According to waste procedures:

- Chemical waste
- Methane
- Cells and cell-media with antibiotics must also be disposed as risk waste.

- Other waste used in this SOP can be disposed in a waste bin right away.

### 9. REFERENCES

MSDS  
ECOonline